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Myxozoa in high Arctic: Survey on the central part of Svalbard archipelago

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ABSTRACT

Myxosporeans (Myxozoa), microscopic metazoan parasitic organisms, are poorly studied in the Arctic region. Our survey of benthic and pelagic fish (n = 234) collected in Isfjorden (Svalbard, Norway) together with detailed morphological and molecular examination revealed the presence of nine myxosporean species. We compared observed myxosporean diversity with diversity documented in regions close to the Arctic and revealed that water depth rather than geographic distribution is an important factor influencing myxosporean fauna.

We describe three new myxosporean species: *Zschokkella siegfriedi* n. sp. from kidney of *Boreogadus saida*, *Parvicapsula petuniae* n. sp. from the urinary bladder of *Gymnocanthus tricuspis*, and *Sinuolinea arctica* n. sp. from the urinary bladder of *Myoxocephalus scorpius*. We characterise *Latyspora*-like organism from kidney of *Clupea harengus*. We provide new data for *Ceratomyxa porrecta*, *Myxidium gadi*, *Myxidium finnmarchicum*, *Schulmania aenigmatosa*, and *Parvicapsula irregularis* comb. nov. The phylogenetic analyses including the newly obtained SSU and LSU rDNA data revealed that most of the species studied cluster in the marine urinary clade within the marine myxosporean lineage. Newly obtained sequences including the first molecular data for the member of the genus *Schulmania*, substantially enriched the *Zschokkella* subclade. *C. porrecta* and the two *Myxidium* species cluster within the *Ceratomyxa* and marine *Myxidium* clade, respectively.

Newly described species, *Z. siegfriedi* n. sp., was revealed to be morphologically indistinguishable but genetically diverse from *Zschokkella hildae* known from numerous gadid fish. Therefore, we consider *Z. siegfriedi* to be a cryptic myxosporean species that might be misidentified with *Z. hildae*. A *Latyspora*-like organism was found to be taxonomically problematic due to its suture line and its distant phylogenetic position from the type species *Latyspora scomberomori* did not allow us to assign it to the genus *Latyspora*. Based on an increased taxon sampling and SSU + LSU rDNA-based phylogeny, evolutionary trends within the marine urinary clade are investigated.

1. Introduction

Arctic ecosystems draw our attention due to their rapid responses to climate change (Post et al., 2009). The Arctic region can be defined as north of the Arctic Circle, and consists the Arctic Ocean, northern part of Eurasia and North America, Greenland, Iceland, Svalbard archipelago etc. The Arctic can be divided into the Low Arctic and High Arctic, according to various environmental and biological characteristics. The Svalbard archipelago is located in the High Arctic. The Arctic Ocean is the most extreme ocean in regard to the seasonality of light and its seasonally fluctuating ice cover. In general, species richness is lower in the Arctic than at lower latitudes and is to some degree constrained by biotic and abiotic mechanisms that define species occurrences and associations (Hoberg and Kutz, 2013). Furthermore, species richness tends to decline from low to high Arctic (Payer et al., 2013). Low numbers of host species is usually correlated to low numbers of parasites. Moreover, water temperature may influence transmission dynamics and parasite development (e.g. Kerans et al., 2005). Arctic fjords in the west coast of the Svalbard archipelago region are exceptional in terms of significantly higher temperatures caused by the Gulf Stream. Variations in the number of parasites were found in morphotypes of threespine sticklebacks living in different temperatures; higher numbers of parasites were found in the morphotype from the deep-cold water habitat compared to

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two warmer water dwelling morphotypes in the same Iceland lake (Karvonen et al., 2013). The enriching effect of warmer temperatures on higher abundance and species richness of ectoparasites was demonstrated in more than 100 fish hosts. This effect is not an artefact, but rather an indication of the importance of temperature in the diversification of fish parasites in the tropics (Poulin and Rohde, 1997).

Myxosporean fauna has been poorly studied in the Arctic region. One of the most parasitologically and ecologically studied marine fish with high economical importance occurring in sub-Arctic and Arctic waters is the Atlantic cod *Gadus morhua* (Hemmingsen and MacKenzie, 2001; Perdiguero-Alonso et al., 2008). Apart from a number of protozoan and metazoan parasites (mostly helminths), 11 myxosporean species have been found in Atlantic cod (Hemmingsen and MacKenzie, 2001; Køie et al., 2007a; Holzer et al., 2010). A survey of parasite fauna of Atlantic cod revealed relatively rich and abundant regional macroparasite fauna dominated mostly by generalist parasites with Arctic-Boreal distribution in six localities in the North East Atlantic (Perdiguero-Alonso et al., 2008). These high-level fauna comparisons suggest that differences in the feeding behaviour of cod amongst localities which could affect the prevalence and abundance of parasite species. Kerans et al. (2005) found that water temperature influenced parasite development rates and was a primary determinant for the release of actinospores of the myxozoan *Myxobolus cerebralis* in strains of its definitive host *Tubifex tubifex*. In addition to latitudinal temperature gradients, sea depth is an important factor for parasite ecology. Low parasite richness was reported in different meso- and bathypelagic fishes in comparison to benthopelagic species in the Arctic Ocean (Klimpel et al., 2006).

This study is focused on the Myxozoa, microscopic metazoan parasites characterised by simplified bodies. Evolutionary history of the Myxozoa has been questioned until recent molecular evidence proved the cnidarian origin (Jiménez-Guri et al., 2007; Holland et al., 2011). Myxozoans infect various organs in the vertebrate, mainly fish, hosts: coelozoic species multiply in the cavities of body organs (gall bladder, urinary tract, renal corpuscles etc.) whereas histozoic species are intercellular in various tissues (liver, skin, kidney, testes etc.). The phylum Myxozoa is divided into two classes: Malacosporea with only three described species and Myxosporea with the overwhelming majority of the myxozoan species. Until now, approximately 2310 myxosporean species assigned to 60 genera have been described (Morris, 2010). Myxosporean genera are characterised by the morphology of the spore: spore shape, number of spore valves and polar capsules (PCs), and position of suture lines towards the PCs are considered the main taxonomic features. However, many myxospore morphological features are not synapomorphic since great discrepancies were found between the classic taxonomic approach and the phylogenetic relationships (Holzer et al., 2004; Fiala and Bartošová, 2010).

Myxosporeans form two main phylogenetic lineages according to host habitat, i.e. marine and freshwater (Fiala, 2006), plus a recently revised third basal sphaerosporid lineage (Bartošová et al., 2013). The marine lineage exclusively consists of marine species with the exception of *Ceratomyxa shasta*. There are five clades within the marine lineage: the marine *Myxidium* clade, the *Ceratomyxa* clade, the *Enteromyxum* clade, the *Kudoa* clade and the marine urinary clade divided into the *Parvicapsula* and *Zschokkella* subclade (Fiala, 2006; Bartošová et al., 2011). With the exception of the *Enteromyxum* clade, the remaining clades include non-monophyletic genera. The clustering of species in particular clades follows tissue tropism criterion rather than myxospore morphology (Holzer et al., 2004; Fiala, 2006). The marine urinary clade is typical in this respect: phylogenetically closely related myxosporeans of the genera *Parvicapsula*, *Gadimyxa*, *Sphaerospora*, *Sinuolinea*, *Latyspora*, and *Zschokkella* differ in spore morphology but predominately infect the excretory tract (Bartošová et al., 2011). However, some species of the *Parvicapsula* subclade also infect other sites such as the epithelium of the gall bladder, the intestine, the pseudobranchs and testicles. The monophyly of the genus *Parvicapsula* was disrupted by clustering of *Gadimyxa* spp. with parvicapsulids as well as by the sister relationship of *P. minibocornis* and *Sphaerospora testicularis* (Køie et al., 2007a; Bartošová et al., 2011). The *Zschokkella* subclade contains species of the polyphyletic genus *Zschokkella* including its type species *Z. hildae* as well as type species of the genera *Latyspora* and *Sinuolinea* (Bartošová et al., 2011; Dyková et al., 2013). The *Zschokkella* subclade is characterised by species with high variability in myxospore shape with the position of PCs ranging from set at opposite ends of the spore, to directly next to each other.

This paper attempts to characterise myxosporean fauna on the Svalbard archipelago: (i) detailed morphological and molecular characterization of myxosporean species; (ii) phylogeny and evolutionary trends; (iii) comparison of parasite diversity from the Arctic with other regions.

2. Material and methods

2.1. Fish hosts

Eight species of teleost fish were collected in part of the Billefjorden, Isfjorden, Petunia Bay (78° 69′ N, 16° 53′ E) in the central part of Svalbard archipelago during the summer season (July and August 2011). A total of 234 individuals of 8 fish species from 7 families were dissected. Families, namely Cottidae: *Myoxocephalus scorpius* (Linnaeus, 1758) ($n = 98$), *Gymnocanthus tricuspis* (Reinhardt, 1830) ($n = 22$); Clupeidae *Clupea harengus* Linnaeus, 1758 ($n = 66$); Osmeridae: *Mallotus villosus* (Müller, 1776) ($n = 16$); Gadidae: *Boreogadus saida* (Lepechin, 1774) ($n = 14$); Pleuronectidae: *Hippoglossoides platessoides* (Fabricius, 1780) ($n = 9$); Myctophidae: *Lumpenus lampretaeformis* (Walbaum, 1792) ($n = 8$); and Salmonidae: *Salmo salar* Linnaeus, 1758 ($n = 1$). Fish were caught using gillnets in littoral habitat (maximum depth of gillnets was 40 m). After euthanasia all organs were checked for the presence of the Myxozoa in squash preparations by light microscopy (Olympus BX 53). Contents of gall and urinary bladders were examined fresh, under cover slips, on slides covered with a thin layer of 1% agar. In some cases we failed to obtain samples of gall and urinary bladders and the missing data are considered in prevalence records within species descriptions. A DNA sample of *Parvicapsula minibicornis* was obtained from the kidney of *Oncorhynchus nerka* (Walbaum, 1792) in Cultus Lake (British Columbia, Canada).

2.2. Myxosporean collection and documentation

Pictures of fresh spores were made using an Olympus BX 53 microscope with Nomarski differential interference contrast equipped with an Olympus DP72 digital camera. Measurements of spores were analysed in ImageJ v.1_44p (Wayne Rasband, http://imagej.nih.gov/ij). Measurements are presented in micrometres. Means, standard deviation (SD) and range in the parentheses were calculated for each spore dimension. Range of plasmodia size is followed by mean and median in parentheses. For examination of fine structure of myxosporean spores and plasmodia by transmission electron microscopy (TEM), whole urinary bladders as well as samples of their contents and kidney tissue were fixed in cacodylate buffered 3% glutaraldehyde at 4 °C, rinsed in 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide. After graded acetone dehydration, the samples were embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEOL JEM 1010 electron